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Effect of soil properties on availability and mobility of Selenium

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EDTA: Ethylenediaminetetraacetic acid
S: Sulphur
Se: Selenium
CaCl₂: Calcium chloride
Abstract

The effect of soil properties on selenium (Se) mobility and availability to wheat was investigated to develop strategies for more cost-effective and environmentally safe Se supplementation. This was done using wheat (*Triticum aestivum*) plants grown on sandy loam soils each amended with 5% inorganic (CaCO$_3$) and organic (compost, cow manure and pig manure) treatments and spiked with 1 mg kg$^{-1}$ of sodium selenate or sodium selenite. The control (reference treatment) consisted of an extra treatment spiked with 1 mg kg$^{-1}$ barium selenate. The influence of soil ageing on Se mobility and plant availability was also assessed. Firstly the soil was characterised, including three soils extractions using CaCl$_2$, EDTA and aqua regia. Also pore water was collected using Rhizon samplers. The results showed that plants grown on selenate spiked soils had higher Se concentrations compared to plants grown on selenite spiked soils. In the soils amended with organics, the Se concentration in plants grown on soils spiked with selenate or selenate was lowest when using cow and pig manure than when compost is used. Liming of the soil led to an increased Se uptake by plants when applied to soil in form of selenate. The uptake of Se supplied to the plants in the form of sodium selenate was found to be higher than barium selenate. Considering the variability in Se uptake by wheat, there is a risk of environmental contamination especially of ground water when Se is applied to the soil in the form of selenite and on soils which are amended with cow and pig manure irrespective of the form in which Se is applied to the soil. However, organic amendments like compost and cow manure may also supplement the Se concentration available for plant uptake when used as soil amendments.
1. Introduction

1.1 Background

Essential trace elements play a vital role in life considering their use in the synthesis of biomolecules on which human and animal life depends. Selenium is an example of an essential trace element which since the 1970s was well recognized as an essential micronutrient for human and animal health because of its biological role as a component of the antioxidant enzyme glutathione peroxidase (GSH-Px; EC. 1.11.1.9), which scavenges hydrogen peroxide and lipid hydroperoxides to prevent oxidative damage in body tissues (Rotruck et al., 1973). Aside the essentiality of Se as a micro nutrient, it is also considered as a toxic element to humans and animals (Ohlendorf, 1989). The toxic effects are mainly as a result of excess intake or failure of the mechanism regulating its concentration.

Dietary Se levels required by animals are within the range 0.05-0.3 mg Se kg\(^{-1}\) dry matter (DM), depending on the animal species and the level of vitamin E in the feed (Ohlendorf, 1989; Mayland, 1994; Oldfield et al., 1994). In cereals and cereal products, Se concentration ranges from 10 to 550 mg kg\(^{-1}\) on a fresh weight basis (FAO, WHO, 2001). The fact that Se has a very narrow range between dietary deficiency and toxicity (40 µg d\(^{-1}\) and 400 µg d\(^{-1}\), respectively; Fordyce, 2007) makes it necessary to control its intake by humans and other animals, and hence important to understand the relationship between environmental exposure and health. Thus, increasing the Se content of plants without exceeding the toxic levels for humans and animals is of major concern considering the Se related health effects.

Through time, low Se concentrations in certain regions of the world have resulted in various diseases such as the alkali disease, white muscle disease, heart disorder (Keshan disease) and bone and joint condition (Kashin-Beck disease) in various parts of China (Fordyce, 2005). Contrary to this, there are also regions in the world with very high soil Se concentrations. Due to this variability, Se intake by humans will also show great variability based on the location. This varies from 7-11 mg d\(^{-1}\) in the Keshan disease area, to several thousands mg day\(^{-1}\) in the selenosis area in the Enshi county of central China. In Europe, Se intake by adults is in the range of 30-100 mg d\(^{-1}\) (Combs, 2001).
In Se rich and deficient areas, human Se uptake and health effects are dependent on Se uptake by food crops from the soil, which in turn depends on the plant availability of different Se forms that may occur. Selenium forms in the environment include selenide, elemental Se, selenite, selenate and organic Se (e.g. selenomethionine). Amongst these, selenate has been reported to be the most bioavailable form for plants (Adriano, 1986). The plant bioavailability also depends on soil factors which include pH, redox condition, soil texture, mineralogy, organic matter content and the presence of competitive ions (Fordyce, 2007).

It has been estimated that between 0.5 and 1 billion people globally may have inadequate intakes of Se, and these include populations in developed countries such as Western Europe (Combs, 2001). It is therefore of paramount importance to develop strategies to improve Se uptake in such countries. It is therefore of paramount importance to develop strategies to improve Se uptake in such countries, without inducing toxicity for the human population or the environment.

1.2 Rationale

Higher plants differ widely in their ability to accumulate Se (Rani et al., 2005; Zhang et al., 2007). Mayer (1997) reported that dietary Se repletion may reduce cancer incidence in people at high risk who live in areas with low soil Se. In an attempt to supplement the Se intake by humans several strategies were put into practice which include foliar application, inorganic fertilization, seed treatment and soil incorporation of fly ash, municipal refuse incinerator ash, or sewage sludge (Logan et al., 1987; Mayland et al., 1989; Arthur et al., 1992).

Wheat (*Triticum aestivum*) and its products were reported to be among the most effective crops for Se supplementation (Lyons et al., 2003; Hawkesford and Zhao, 2007). Fox et al. (2005) compared the efficiency of Se absorption from three food sources by humans using intrinsic labelling with the stable isotopes $^{77}$Se or $^{82}$Se. They found that Se absorption was significantly higher for wheat (81%) and garlic (78%) compared to fish (56%). Due to the high uptake of Se, wheat would be a good choice for enhancing intake by humans.

It is worth considering that different types of fertilisers may influence Se bioavailability and subsequent uptake by wheat. This constitutes the backbone of this study in which the effect of
liming and using different organic amendments (compost, cow manure, pig manure) on the Se mobility, availability and uptake by wheat will be investigated.

1.3 Objectives

The main goal of this study is to explore how different amendments affect Se availability and mobility in the soil, and its uptake by plants.

The specific objectives are:

1) To investigate the effect of lime, compost, pig and cow manure amendments on Se mobility, availability and uptake by wheat in a sandy loam soil spiked with 1 mg Se kg\(^{-1}\) in the form of selenate or selenite.

2) To investigate the impact of soil ageing after Se fertilisation on Se mobility, availability and uptake by wheat and how these ageing effects depend on soil amendment and type of Se fertilizer.

This will enable us to obtain insights into the influence of organic and inorganic soil amendments and soil ageing on Se mobility, availability and uptake by wheat, leading to more cost–effective and environmentally safe Se supplementation.
2. Literature review

2.1 Occurrence

Selenium is a naturally occurring metalloid, essential to human and animal health in trace amounts but harmful in excess (Fordyce, 2007). Its essentiality to plants still remains controversial (Minorsky, 2003). Selenium is found in water, air and soil at various concentrations as a result of natural and anthropogenic activities.

Geology exerts a fundamental control on the concentration of Se in soils on which we grow the crops and animals that form the human food chain. Hence, with the varying geological conditions worldwide, Se-rich and Se-deficient areas occur, and thus, varying concentrations in crops and animals. In general terms, sedimentary rocks contain greater concentrations of Se than igneous rocks, with exceptionally low levels of 0.1 mg kg\(^{-1}\) in sedimentary rocks like limestone and sandstones (Fordyce, 2005). Exceptions to this low value in sedimentary rocks are observed in clays and shales (Neal, 1995). Also, very high concentrations have been observed in phosphatic rocks probably reflecting similarities between organically derived phosphate (PO\(_4^{3-}\)) and selenate (SeO\(_4^{2-}\)) anions (Fleming, 1980; Jacobs, 1989; Nriagu, 1989; Neal, 1995). The world mean concentration of Se in soils is 0.4 mg kg\(^{-1}\) (Fordyce, 2005).

The anthropogenic sources are a result of using Se and its compounds for a variety of purposes, including red pigmentation in glass and ceramics, use in photocopiers, catalysts, antiseptics against dandruff and fungi in pharmaceutical industries, extraction and processing of copper and lead, combustion of coal and petroleum fuels, use of phosphate fertilizers, sewage sludge and manure on land, etc.

Since plants represent a major uptake route of this element by humans an in-depth study of environmental biogeochemistry controls on distribution and mobility of Se is a prerequisite to understanding Se-related health effects. These health effects are not only dependent on the total soil Se concentration but also on the bioavailable forms that can be readily taken up by plants (Fordyce 2005).

Moxon (1937) was the first to examine the relationship between geology, soil Se concentrations, uptake into plants, and health outcome in animals. In his study, he reported
that soils capable of producing Se-rich vegetation toxic to livestock were found over black shale and sandstone deposits in the Great Plains of the United States. Subsequent studies (Muth and Allaway, 1963) focusing on Se-deficiency related diseases in animals led to production of one of the first maps of the Se status of soils, vegetation and animals, and the establishment of the classic Great Plain seleniferous soil types.

2.2 Forms of Occurrence of Se

The major forms of Se in the environment include selenide, elemental Se, selenite, selenate and organic Se (e.g. selenomethionine) (Adriano, 1986). These, their oxidation states and chemical forms are shown in Table 2.1.

Table 2.1. Chemical forms of Se in the environment.

<table>
<thead>
<tr>
<th>Oxidative state</th>
<th>Chemical forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se(^{-2})</td>
<td>Selenide (Se(^{2-}), HSe(^{-}), H(<em>2)Se(</em>{aq}))</td>
</tr>
<tr>
<td>Se(^{0})</td>
<td>Elemental Selenium (Se(^{0}))</td>
</tr>
<tr>
<td>Se(^{4+})</td>
<td>Selenite (SeO(_3)^{2-}, HSeO(_3)^{-}, H(_2)SeO(_3aq))</td>
</tr>
<tr>
<td>Se(^{6+})</td>
<td>Selenate (SeO(_4)^{2-}, HSeO(_4)^{-}, H(_2)SeO(_4aq))</td>
</tr>
<tr>
<td>Organic Se</td>
<td>Selenomethionine, Selenocysteine</td>
</tr>
</tbody>
</table>


The different oxidation states determine the forms available for plant uptake. Of these, selenate is the main bioavailable form followed by selenite. This is explained by the chemical similarity of sulphur (S) and Se in terms of their oxidation states. Hence, plants can easily take up selenate through the sulphur pathway (Dahillon and Dahillon, 2004) and therefore represent a major route through which humans obtain their safe concentration of Se. Selenate is taken up by plant roots via the high affinity of sulphate transporters (Terry et al., 2000). There is little know about the selenite uptake mechanism by plants but it was suggested that selenite is taken up by plant roots via passive diffusion (Shirft and Ulrich 1969; Arvy, 1993). Selenite is strongly adsorbed by soil minerals and thus has a low bioavailability to plants, whereas selenate is much more bioavailable (Gissel-Nielsen et al., 1984). Li et al. (2008) reported that in wheat roots selenite can be taken up by phosphate transporters via a metabolically-dependent active process. Selenite, upon uptake, is rapidly assimilated into organic forms in roots and has a low mobility in xylem transport. On the contrary, selenate is
not readily assimilated into organic forms, but is highly mobile in xylem transport. Furthermore, selenite appears to inhibit the transport of selenate in the xylem.

It is estimated that perhaps 50% of Se in some soils is held in organic compounds (Fordyce, 2005). Selenomethionine has been extracted from soils and has been found to be two to four times more bioavailable to plants than inorganic selenite while selenocysteine is less bioavailable than selenomethionine (Jacobs, 1989; Mayland, 1994; Neal, 1995).

2.3 Factors determining Se uptake by plants

Selenium mobility and uptake by plants and animals are dependent on its bioavailability. This is dependent on a number of bio-physical parameters which include pH, redox condition, chemical form of the element or speciation, soil texture, mineralogy, organic matter content and the presence of competitive ions in the soil. Hence an understanding of these parameters is essential for the prediction and remediation of health risks from Se as soils that contain adequate total Se concentrations can result in Se deficiency if the element is not in a readily bioavailable form (Fordyce, 2007).

2.3.1 Redox potential and pH

Although transformation between selenite and selenate is a slow process, both forms may co-exist under the conditions prevalent in agricultural soils (Geering et al., 1968). The oxidising conditions and pH will therefore determine the forms available for plant uptake. Selenite is the dominant Se species under moderate redox soil conditions, where it is strongly adsorbed by iron and aluminium oxides and hydroxides, resulting in limited plant availability and a low potential for leaching (Elrashidi et al., 1987). The same author also reported that selenate is the dominant species under high redox soil conditions. Under this high redox condition, selenate is weakly adsorbed by electrostatic forces of attraction (Jackson and Miller, 1999), resulting in high plant availability and potential for leaching. Adsorption of these Se forms in soils also depends on the soil pH. In acidic and neutral soils, selenite is insoluble while in neutral and alkaline soils, selenate is more available for plant uptake (Fordyce, 2005). This has been confirmed in experimental studies with 10 times more uptake of Se in Se-selenate enriched soils compared to Se-selenite enriched soils (Jacobs, 1989; Neal, 1995).
Thermodynamic calculations also show that selenate should be the predominant form in alkaline and well-oxidized soils (\(pe + \text{pH} > 15\)), and selenite in well-drained mineral soils with pH from acidic to neutral (\(7.5 < pe + \text{pH} < 15\)) (Elrashidi et al., 1987).

Elemental Se, selenides and selenium sulphide salts tend to exist in reducing, acidic and organic rich environments only, which due to their low oxidation potential and solubility, these species are not bioavailable to plants (Fordyce, 2005). Therefore, in any study of the selenium status of soil, consideration of the likely bioavailability is important. Of the several different chemical techniques used to assess bioavailability, the most widely accepted indicator is the water-soluble Se content (Jacobs, 1989; Tan, 1989; Fordyce et al., 2000b). The carbonate levels in soils have also been reported to play an important role in buffering acidic soils to a neutral and slightly alkaline pH (Singh et al., 1998; Vandecasteele et al., 2002b). Their effect on soil pH will also determine Se availability in soils.

### 2.3.2 Organic Matter

The influences of organic matter on Se bioavailability are two fold: the binding of Se by soil organic matter and the decomposition of Se containing organic matter, releasing Se in either a bioavailable or non-bioavailable form.

A reduction in plant Se with increased organic C in soils was reported by Bisbjerg and Gissel-Nielsen (1969) and Levesque (1974). The main mechanism is due to fixation by organometallic complexes. This has been confirmed in experimental set-ups where the uptake of selenate from organic rich soils is 10 times smaller than in mineral soils (Joacobs, 1989; Niels, 1995). This was further confirmed in a pot experiment by Johnsson (1991), during which it was revealed that an increase in organic matter content decreased the Se concentration in wheat grains from 1350 to 160 µg kg\(^{-1}\). An indication of the effect on bioavailability is portrayed in an experiment by Neal and Sposito (1991) who investigated the effects of organic carbon amendments on selenate immobilisation in soils and found that addition of dextrose resulted in a transformation of a large portion (64-90%) of added Se\(0_4^{2-}\) into organically associated forms. A similar study on Se bioavailability was carried out by Dhillon et al. (2010), who reported that incubating naturally occurring selenium forms with organic amendments led to a substantial decrease (20%-26%) of easily available (water-
soluble and extractable) forms of Se and a corresponding increase of (13%-62%) in the less available (organic matter and metal oxide bound) Se forms.

Another mechanism illustrating the effects of organic amendments to soils on the reduction of Se bioavailability is the reduction of oxidised forms to elemental Se (Tokunaga et al., 1996). In seleniferous soils, the accumulation of Se by canola and wheat has been reported to decrease considerably when treated with organic amendments (Sharma et al., 2011).

Organic matter does not decrease bioavailability but may serve as a supplementary source of Se to plants. Zawislanski and Zavarin (1996) using sequential extraction techniques suggested that a large portion of total Se in soils can be in the organic fraction. They concluded that the release of low molecular-weight Se compounds during organic matter decomposition may eventually be a source of Se for plant uptake. Information on plant availability of Se from different seleniferous plant residues and animal manures is thus needed to better understand Se budget in the environment and to determine strategies for lowering Se concentrations in soils and to supplement animal diets (Ajawa et al., 1998).

Organic amendments may also introduce large amounts of low-molecular weight organic acids to soils which strongly affect nutrient mobility and plant availability (Baziramakenga and Simard, 1998). Selenium retention by various organic manures may depend upon the type of monocarboxylic and multicarboxylic organic ligands released into the soil during decomposition (Sharma et al., 2011). Dissolved polysaccharides such as starch, amylopectin, water-soluble lignin, cellulose acetate, chitosan and carboxymethylcellulose in soils can immobilise Se by forming stable compounds with selenate (Ferri and Sangiorgio, 1999). A characterisation of the biochemical nature of organic amendments was thus proposed by Sharma et al. (2011) to better understand the differences in their efficiency of reducing Se bioavailability.

2.3.3 Soil type

Soils originate from rocks via the process of weathering. Excessive soil Se concentrations (greater than 3 mg kg\(^{-1}\)) occur in areas of North America, China and Ireland, whereas deficient soil Se concentrations (less than 0.125 mg kg\(^{-1}\)) occur in Siberia, New Zealand and
the Keshan area of China (Broadley et al., 2006). These are due to differences in the parent material, which represents an important factor in determining the Se concentrations in soils (Spadoni et al., 2007). Seleniferous soils therefore originate from a seleniferous parent material. With such high soil concentrations, one may expect to find high concentrations in plants and animals. Seleniferous soils with total Se ranging from 0.50 to 4.55 mg kg\(^{-1}\) have also been identified in Hoshiarpur and Nawanshehar districts of Punjab, India (Dhillon and Dhillon, 1991). These are alkaline and calcareous in nature with a major portion of the total Se existing in readily available forms (Dhillon and Dhillon, 2004). Soils with a Se concentration greater than 0.5 mg kg\(^{-1}\) have been found to produce vegetation absorbing Se at levels toxic for animal consumption (Dhillon et al., 1992). An exception to this was observed in Hawaii with up to 20 mg kg\(^{-1}\) total Se but without any effect on vegetation and livestock because the element was held in iron and aluminium complexes in the humid lateritic soils of that region (Oldfield, 1999). In Se deficient soils, the Se content of livestock is supplemented through application of Se to soils by farmers (Gissel-Nielsen et al., 1984; Rimmer et al., 1990).

An important source of Se in diets is wheat grain and its products, which contributes 22 % of dietary Se in countries like the UK (Rayman, 2000). However concentrations of Se in European wheat are generally low (Hawkesford and Zhao, 2007), likely to be caused by low Se supply from the soil.

### 2.3.4 Presence of other ions

A significant positive correlation (r > 0.84) has been reported between S and Se content for raya, rice, maize and wheat (Rani et al., 2005). Sulphate and phosphate ions have an influence on Se uptake in soils by competing for fixing sites in the soil and plants. Sulphate inhibits Se uptake by plants with greater effect on selenate compared to selenite. A synergistic relation interaction between selenate and sulphate was reported by Smith and Watkinson (1984) and Mikkelsen et al. (1988a, b) who observed that elevated concentrations of Se in the root zone might increase the plant S accumulation when sulphate concentrations are low in the root zone. Contrary to this, an antagonistic relationship between sulphate and selenate was also reported, firstly, by Hurd-Karrer (1938) and subsequently confirmed by others: Shrift, (1954); Gissel-Nielsen (1973); Spencer (1982); Dhillon and Dhillon (2000);
Terry et al., (2000); Adams et al., (2002). Thus, the presence of abundant sulphate in the root zone of plants could reduce the Se uptake by plants resulting from the antagonistic interaction between sulphate and selenate ions for plant uptake or may simply reflect a dilution of plant Se due to increased plant growth as a result of S addition to S-deficient soils (Mikkelsen et al., 1989). This effect also implies that the main form of Se taken up by wheat is selenate, because uptake of selenite is not inhibited by sulphate (Li et al. 2008). The threshold S concentration at the root zone determining the antagonistic or synergistic effect is not known (Rani et al., 2005).

Selenate does not accumulate in the roots of plants when taken up (Läuchli, 1993), rather it is translocated to the chloroplasts where it follows the S assimilation pathway (Terry et al., 2000). This substitute for S leads to the formation of selenoamino acids which enhances ethylene production (Konze et al., 1978). This can in turn modify the membrane lipid composition (Thompson et al., 1982), increase membrane permeability and K$^+$ leakage (Vangronsveld et al., 1993).

Examples of these selenoaminoacids include selonomethionine and selenocysteine. Selenocysteine may be incorporated into proteins in place of cysteine which affects the formation of disulfide bridges. This alters the protein structure, leading to inactivation of the protein, hence reduced enzyme activities and eventual poisoning of the plant (Brown and Shrift, 1982). This was confirmed by Sharma et al. (2008) who reported deterioration in the quality of wheat and oilseed rape based on lipid and protein concentrations due to Se accumulation. Cartes et al. (2006) also reported a decrease in Se incorporation into soluble proteins in response to increased amounts of S applied to soil. Phosphate addition to soil increases Se uptake by plants as they readily adsorb on soil displacing selenite from fixation sites making it more bioavailable. Increased phosphate concentration also dilutes the Se content of plants by inducing increased plant growth (Jacobs, 1989; Mayland, 1994; Neal, 1995).

2.3.5 Plant Species

Plants vary considerably in their physiological response to excess Se in the growth medium (Terry et al., 2000), hence determining the Se related health problems in humans and animals
WHO, 1987; Neal, 1995). Appreciable differences in the uptake and transport of various Se compounds in plants have been reported by Yu and Gu (2007). In contaminated soils cultivating Se accumulating plants has been suggested for lowering the Se levels (Banuelos, 2001). Brassica species have been deployed in phytoremediation of Se in soils. These were reported to have a potential for reducing up to 40% of total soil Se under greenhouse conditions and up to 20% under field conditions after one growing season (Banuelos, 2000). In seleniferous regions, plants belonging to the cruciferae family absorb much greater amounts of Se compared to those belonging to the graminae family (Dhillon and Dhillon, 1997).

Toxicity of Se can thus be related to the differences in ability of different plant species to accumulate the element based on the environmental concentrations. Rosenfield and Beath (1964) were the first to classify plants in three groups based on ability of Se uptake:

- Se-accumulator plants which grow well in high Se soils and can absorb more than 1000 mg kg$^{-1}$;
- Secondary Se-absorbers which absorb between 50-100 mg Se kg$^{-1}$;
- Low absorbers (mostly grains and grasses) which accumulate less than 50 mg Se kg$^{-1}$.

The variability in absorption is due to differences in metabolism of Se. Plants contain many different Se compounds and the main form in non-accumulator species is protein-bound selenomethionine. However, selenocysteine and selenonium have also been reported (Jacobs, 1989; Niels, 1995). In accumulator plants, Se metabolism is based on water-soluble non-protein forms such as Se-methylselenomethionine. The exclusion of Se from proteins in accumulator plants is thought to be the basis of Se tolerance (Jacobs, 1989; Niels, 1995). Plants also have the ability to reduce selenate to elemental Se$^0$ and selenide Se$^2$- forming volatile organic compounds like dimethylselenide and dimethylselenide, which are all released during respiration giving rise to a “garlic” odour characteristic of Se-accumulating plants (Mayland, 1994).

The mechanism of absorption also influences the concentration in the plant. Both selenate and organic Se compounds are absorbed by plant roots through active processes whereas selenite uptake seems to be through passive diffusion (Terry et al., 2000; White et al., 2004). Despite these various mechanisms of coping with Se, plants can also suffer toxicity via the following ways (Jacobs, 1989; Rergusson, 1990; Mayland, 1994; Wu, 1994):

1) Se competes with essential metabolites for sites in the plant biochemical structure;
2) Se may replace the essential ions, mainly the major cations (e.g. iron, manganese, copper and zinc);
3) Selenate can occupy the sites for essential groups such as phosphate and nitrate;
4) Se can be incorporated into analogues of essential sulphur compounds in plant tissues.

Other effects have been reported in plants. As an example, a decrease in plant growth from excessive Se has been reported (Wan et al., 1988; Mikkelsen et al., 1988a). In wheat plants growth and metabolism is affected by soil concentrations greater than 2 µg g\(^{-1}\) (Yang et al., 1983). Hartikeinen et al (2000) also reported that aside the effects on plant metabolism, at low concentrations Se acts as an antioxidant in plants inhibiting lipid peroxidation. At high concentrations (> 10 µg g\(^{-1}\)), it acts as a pro-oxidant encouraging the accumulation of lipid peroxidation products leading to high yield losses. In general, root crops contain higher Se concentration than other plants and plant leaves often contain higher concentrations than the tuber. Also, crop species grown in very low Se-soils show very little difference in uptake and changing the type of plant has no significant impact on the Se content of crops (Jacobs, 1989).

Loss of dry matter yields have also been reported to be associated with an increase in the Se content of plant tissues. This was attributed to the substitution of Se in place of S in essential organic compounds leading to the disruption of normal biochemical reactions and enzyme functions (Anderson and Scarf, 1983). Dry matter production in raya, maize and wheat was reported to be adversely affected by high levels of Se in the soil (Rani et al., 2005). Another toxicity symptom occasionally seen in wheat grown on Se toxic soils is snow-white chlorosis with pink colouration on the lower side of the leaves (Rosenfeld and Beath, 1964; Dhillon and Dhillon, 2000). Sharma et al. (2011) reported a decrease in the protein and lipid concentrations in rape grains due to Se accumulation at high levels. This was attributed to the incorporation of Se in nonprotien aminoacids such as Se-methylselenocysteine, i.e., replacement of S in cysteine and methionine (Nigam and McConnell, 1973), thereby making these amino acids partly unavailable for protein synthesis. The decrease in lipid concentration due to Se accumulation was thought to have resulted from a decrease in the activity of fatty acid synthetase and acetyl CoA carboxylase, which are the enzymes having SH groups at the active site and most possibly incorporate Se in place of S (Terry et al., 2000).
In Se deficient areas, biofortification of Se in crops, through Se fertilization, has been practised in many countries of the world such as Finland since the mid-1980s (Eurola et al., 1991). Breeding or genetic manipulation of crops with enhanced Se uptake has been proposed as an effective way of increasing human Se intake through plants (Lyons et al., 2003; Broadley et al., 2006). Wheat plants have been reported to accumulate Se to levels safe for human and animal consumption at concentrations lower than 3 µg Se g⁻¹ of soil though a significant reduction in dry matter yield was observed for plants grown on soils with soil Se concentrations greater than 4 µg g⁻¹ (Rani et al., 2005). Plants like canola and tall fescue grown on soils amended with seleniferous or organic materials accumulate Se below levels toxic to humans (Ajawa et al., 2005).

2.3.6 Microbial activity

Volatileisation of Se in soils is due to microbial activity. Microbes play a major role in the oxidation and reduction of Se. Ajawa et al. (2005) reported a reduction in selenate bioavailability due to stimulated microbial assimilatory reduction of added selenate in soils treated with alfalfa residue or cattle manure. Their results also suggested that a large fraction of Se in seleniferous plant residues incorporated into soils would be volatilized, but only a small fraction of Se in seleniferous cattle manure would be volatilized, and therefore, Se may accumulate in soils. Facultative anaerobes like Enterobacter cloacae SLD1a-1 reported to be capable of reducing selenate and selenite to elemental selenium (Losi and Frankenberger, 1997) was found to volatilise dimethylselenide in the presence of SeO₃ at an optimum pH, temperature and electrical conductivity of 6.5, 35°C and 11 dS m⁻¹, respectively. Microbial activity therefore is of great importance when considering selenium bioavailability in soils.

2.3.7 Influence of soil amendments on Se uptake

Apart from organic amendments, inorganic soil amendments also play a role in determining the Se concentrations of plants. Fertilisation techniques have been reported to play an important role in determining the trace metal content of cereal grains (Pessi et al., 1974). Wheat Se content grown on clay-loamy soils treated with organic manure was observed to be higher than those treated with inorganic fertilizer (Gissel-Nielsen, 1974). Martens and Suarez (1997a) investigated the assimilation of SeO₄²⁻ by alfalfa and mineralization of fresh alfalfa
residue in soil. They found that 25% of the total Se assimilated was present as free Se-amino acids, Se-methylcysteine, selenomethionine, and selenocystine, but 30% of the assimilated Se was mineralized to water-soluble, non-amino acid, selenide-Se, and the remaining organic selenide-Se persisted in protein form. In another study, Martens and Suarez (1997b) found different mineralization and volatilization rates for the selenoamino acids, selenomethionine, and selenocystine in soils amended with animal manure. Their findings suggested that if selenomethionine is the dominant aminoacid Se species in plant residue or animal manure, then most of the Se will be volatilized. However, little volatilization is expected from selenocysteine because it is very unstable in soil and will rapidly oxidize to Selenate, and eventually to Se°.

Gypsum, an inorganic amendment to soils was also reported to increase Se uptake by plants with low absorbing capacity (Bawa et al., 1992; Dhillon and Dhillon, 2000). An exception to this was observed in wheat where the uptake of Se was reported to be reduced after a growth period of 2 years at a gypsum application rate of 0.8 t ha⁻¹. Application of gypsum was thus concluded to aid in effectively reducing the risk of Se over-exposure to animals and humans that depend on the diet based source of Se from wheat and rice grown in the seleniferous region of Punjab (Dhillon and Dhillon, 2000). In white clover, a significant increase in shoot Se concentration when the soil is treated with lime was reported (Mora et al., 2008).

Selenium fertilisation of crops has been shown to significantly enhance Se concentrations in crops grown on soils with low plant available Se concentrations. Finland had successfully applied agronomic biofortification with Se since 1984, with the annual input of Se fertiliser dose being 10 g Se ha⁻¹ (Hartikainen, 2005). However, the fate of Se fertiliser in Finnish soils was not accounted for, considering that less than 20 % of applied selenate was taken up by the crops (Keskinen et al., 2009). It was assumed that selenate is reduced and immobilised in the soil (Keskinen et al., 2009), which is supported by evidence of a limited residual effect. As an example, the application of 10 g Se ha⁻¹ selenate to cereals grown in a Canadian soil was found to have a negligible residual effect after 1 year (Gupta, 1995). However, to monitor the fate of Se fertiliser in soil directly, changes in the concentration and speciation of Se in soil should be determined (Stroud et al., 2010).
2.4 Soil ageing

The transformation of a parent material to clay is due to weathering. This and the decomposition of organic matter over time in soils define the soils age. In soils and sediments, pollution retention is a common phenomenon which increases over time (ageing) explained by the continuous sorption and hence, reduction in bioavailability. This is mainly due to the activity of soil organic and mineral matter through the following mechanisms; adsorption onto mineral surfaces, absorption into flexible (soft) natural organic matter, adsorption on condensed (hard) natural organic matter, diffusion in micro-porous media and encapsulation. These processes as well as physico-chemical driven processes control the sorption and slow release of trace elements in soils, hence determining their bioavailability (Loibner, 2006).

This is illustrated by Tack and Vandecastele, (2008) in a review paper on the cycling and ecosystem impact of metals in contaminated calcareous dredged sediment-derived soils (Flanders, Belgium), who reported that although soil physico-chemical properties favour a low metal bioavailability, pore water concentrations can be elevated, compared to pore water in uncontaminated soils. This probably caused by the dynamics in the soil over time.

2.5 Importance of Se for humans

The intake of Se in countries like the UK is below the reference nutrient intake, which is a cause for concern (Broadley et al., 2006). Humans rely on plants for their Se supply. In plants, the exact use of Se has not been defined while in humans Se serves as a major component of enzymes and glutathion peroxidase (GHS-Px) which acts as an antioxidant preventing oxidative cell degeneration. Through time, the deficiency of this element has been associated with the main cause of diseases like white muscle disease, heart disorder (Keshan disease), and bone and joint condition (Kashin-Beck disease) in various parts of China, and malfunctioning of thyroid hormone metabolism. In animals, the “alkali disease” associated with hair and hoof lose has been reported in areas of the world with seleniferous soils like China (Fordyce, 2007). In humans Se poisoning also causes loss of hair, skin decolouration, nail abnormality and anaesthesia (Yang, 1989).

The use of Se-bearing fertilizers has thus been used to remediate Se deficiency in a number of countries. In certain regions of the world such as the north-eastern parts of Indian Punjab,
elevated levels of Se in agricultural soils have resulted in severe health problems in animals and humans (Dhillon and Dhillon, 1991, 1997).

Due to the complementary role of Se and vitamin E, all Se deficiency diseases in animals are linked with vitamin E deficiency with the exception of neutrophil microbicidal activity reduction and the 5-deiodinase enzymes responsible for the production of triiodothyronine from thyroxine (Fordyce, 2005). Shamberger and Frost (1969) reported an inverse relationship between Se in crops and human blood versus cancer incidence in the United States and Canada depicting the anticarinogetic effect of Se.

Low dietary intakes of Se by humans can thus cause health disorders, including oxidative stress-related conditions, reduced fertility and immune function, and an increased risk of cancer (Rayman, 2000, 2002; Whanger, 2004).
3. Materials and methods

3.1 Experimental Set up

Forty-five kg of homogenised air-dried sandy loam soil was divided into five parts. Of these, one part was used as reference soil while the remaining four were each amended with fresh compost, cow manure, pig manure and lime (CaCO3), respectively. The respective amounts of soil were 11 kg and 8.5 kg for the reference soil and the remaining four amended soils, respectively (Table 3.1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amount of soil/ kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>11</td>
</tr>
<tr>
<td>Compost</td>
<td>8.5</td>
</tr>
<tr>
<td>Cow manure</td>
<td>8.5</td>
</tr>
<tr>
<td>Pig manure</td>
<td>8.5</td>
</tr>
<tr>
<td>Lime</td>
<td>8.5</td>
</tr>
</tbody>
</table>

The compost, cow and pig manure amended soils represent the organic amended soils, while the soil to which lime was added represents the inorganic amended soil. The fresh compost, cow manure and pig manure were oven-dried at 60°C for 48 h to determine their dry weight content (Table 3.2). An amount of fresh weight corresponding with 5% dry weight was added to each soil for the organic amended soils. In the lime amended soil, lime was added at 5% of soil dry weight. In order to ensure homogeneity, the soils with each amendment were mixed in open top plastic containers. No amendments were added to the reference soil.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry weight/ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compost</td>
<td>78.3</td>
</tr>
<tr>
<td>Cow manure</td>
<td>21.2</td>
</tr>
<tr>
<td>Pig manure</td>
<td>28.7</td>
</tr>
</tbody>
</table>

Each amendment was divided into three subgroups, each consisting of 1 kg sampling pots and 500 g pots used for growing wheat.
The pots were filled with 1 kg, 0.5 kg and 0.5 kg of amended or reference soil, respectively, and spiked with Se solution containing a particular selenium salt (10 mg Se L$^{-1}$) to obtain 1 mg Se kg$^{-1}$ soil. The Se solution was applied to the surface after which the soils were mixed in open top plastic containers to ensure homogeneity and the contents were retransferred to their respective pots.

The subgroups each constituted of:

1) a blank treatment which constituted of sandy-loamy soil with and without amendment to which no selenium salt was added.
2) a sodium selenite (Na$_2$SeO$_3$) treatment which constituted of sandy-loamy soil with and without amendment, spiked with 1 mg Se kg$^{-1}$ in the form of sodium selenite.
3) a sodium selenate (Na$_2$SeO$_4$) treatment; which constituted of sandy-loamy soil with and without amendment, spiked with 1 mg Se kg$^{-1}$ in the form of sodium selenate.

In the reference treatment subgroup, an extra treatment was added which constituted the bare sandy loam soil spiked with 1 mg Se kg$^{-1}$ in the form of barium selenate (BaSeO$_4$).

The pots were then labelled indicating the main groups and subgroups. The main groups included:

- Group 1 (Treatment 1): the reference soil
- Group 2 (Treatment 2): the compost amended soil
- Group 3 (Treatment 3): the cow manure amended soil
- Group 4 (Treatment 4): the pig manure amended soil
- Group 5 (Treatment 5): the lime amended soil.

The subgroups were labelled based on the form of selenium spiked to the soil.

These subgroups were again divided into three groups, labelled as T-0, T-1 and T-2. These labels represent the time periods during which they would be used for growing wheat: T-0 represents the pots which were used for growing wheat after 10 days of stabilisation, T-1 and T-2 represent the pots which were used to grow wheat after one and two months of incubation, respectively.

The moisture content in each pot was maintained at 20% by compensating for the deficit with distilled water. A schematic representation of the experimental set up is given in Figure 3.1.
In Figure 3.1, A, B and C represent the 1 kg sampling pots, while the pots labelled with T-0, T-1 and T-2 represent the 0.5 kg experimental pots used for growing wheat. These were then covered with perforated transparent polythene bags sealed with rubber bands in order to minimise evaporation from the pots. They were then kept for stabilisation and after 10 days, the polythene covers were removed from each pot.

The first batch of pots (0.5 kg) was collected from each group (labelled T-0) along with the sampling pots (1 kg). From the sampling pots, 10 g of soil was collected in plastic containers. Moreover, porewater was sampled from these pots using Rhizon soil moisture samplers connected to vacuum tubes. Samples were labelled and the soil samples were kept to air dry while the porewater samples were kept in the freezer at -20°C.

In the 0.5 kg experimental pots, eight wheat seeds were sown. After sowing, these were again covered with perforated transparent polythene bags in order to maintain the moisture content and aid germination. They were then placed in a growth chamber. After one week when germination had taken place, the polythene bags were removed.

The moisture content of these pots was maintained at 20% (by weighing) during the one month growth period. The temperature of the growth chamber was measured using a thermometer indicating maximum and minimum temperatures. After one month of growth, the aboveground biomass of each pot was harvested, and the fresh and dry weights were determined.

After the T-0 samples were harvested, the moisture content was maintained at 20% in the next batch of experimental pots (T-1) and sampling pots. A similar procedure was used as for
the T-0 pots and the same samples were collected. The same procedure was again used for the T-2 pots after the T-1 samples were harvested. The average maximum and minimum temperature during the entire growth period was 27°C and 20°C, respectively.

### 3.2 Soil characterisation

The following analyses were done on the air-dried 10 g soil samples:

- Determination of soil pH, electrical conductivity, organic matter content and cation exchange capacity
- Analysis of selenium in soil extracts, including CaCl2, EDTA and aqua regia extracts.

#### 3.2.1 pH determination

The pH-H2O or the actual acidity was measured in a soil-water suspension. Therefore, 50 mL of distilled water was added to a glass beaker containing 10.00 grams of sediment. The suspension was stirred using a glass rod and then left to equilibrate for 16 hours. After equilibration the pH was measured using a pH electrode (model 520A pH meter, Orion Research Inc., Boston, MA, USA).

#### 3.2.2 Electrical conductivity (EC)

The EC of a solution is an indicator of the amount of soluble salts. This electro-chemical property is based on the fact that the conductance (inverse of the electrical resistance, ohm, Ω) of a solution increases as the concentrations in cations and anions (carriers of electric charges) increase. The EC is expressed in mmho cm⁻¹ or mS cm⁻¹ (mmho = millimho, mho = 1/Ω = Siemens = S) at a temperature of 25°C. The EC is the conductance measured with a conductimetric cell composed of two electrodes having a 1 cm² surface area, separated from each other by 1 cm. The relationship between the amount of dissolved salts and the EC is directly proportional, notably: mS cm⁻¹ x 10 = meq L⁻¹.

Therefore, 50 mL of distilled water was added to a 250 mL erlenmeyer flask containing 10.00 grams of sediment. This was put on a shaker for 1 h, after which it was filtered. The EC was then measured from the filtrate. The EC abbreviated ECe, is used for the soil salinity
classification in relation to the levels of tolerance of different crops. The ECe varies in the order of 0.01 to 10 mS cm\(^{-1}\). Below 2 mS cm\(^{-1}\), the effects of the salinity are generally negligible; above 16 mS cm\(^{-1}\), only a few very tolerant crops are resistant. Details about the procedure are given in the ‘‘Manual for the soil chemistry and fertility laboratory’’ (Van Ranst et al., 1999).

### 3.2.3 Organic Matter

The organic matter content was determined by ashing. Labelled crucibles were dried in an oven at 100°C for 1 h, after which they were put in an excicator to cool down. Thereafter, 3 g of air-dried soil was weighed into the crucibles using an analytical balance and ashed for 2 hours at 550°C in a muffle furnace. After ashing, the crucibles were allowed to cool in an excicator and re-weighed using an analytical balance. The difference in weight of the soil before and after ashing represents the amount of organic matter in the sample.

### 3.3.4 Cation Exchange Capacity (CEC)

The determination of the CEC with ammonium acetate at pH 7 was carried out in three steps, notably;

- saturation of the adsorption complex by the ammonium ion (NH\(_4^+\)) and extraction of exchangeable bases;
- washing of the soil with alcohol in order to eliminate the excess ammonium solution that filled up the pores;
- qualitative analysis of the ammonium after desorption with potassium ion (K\(^+\)).

The exchange reactions can be accomplished by successive extraction, equilibration, centrifugation and decantation (discontinuous method) or by percolation (continuous method). Quantitative analysis of ammonium was done by steam distillation. Details on the procedure are given in the ‘‘Manual for the soil chemistry and fertility laboratory’’ (Van Ranst et al., 1999).

### 3.4 Multi-element soil extracts

Many extraction solutions have been developed that permit simultaneous extraction of a fairly wide range of bioavailable and/or phytotoxic elements and nutrients. The quantities
extracted correspond either to the nutritive state, i.e., to the bioavailable fractions, or the level of pollution in the soils. The bioavailable fractions are extracted using extracting agents, which chemically simulate the root-soil interaction, by acting on solubilization, sorption and complexing reactions which are fundamental to the dynamics of nutrient and bioavailable element transfer. Three extracts were made on the soils; ammonium acetate and ethylene diamine tetracetic acid (EDTA) pH 4.65, CaCl$_2$ (0.01M) and aqua regia, which are amongst those accepted by the major agronomic research organisations (e.g. FAO, USDA, CIRAD). The ammonium acetate-EDTA and CaCl$_2$ (0.01M) extracts were each made from 10 g of soil using 50 mL of each solution. The extracts were stored by plastic containers. Two drops of HNO$_3$ were added to the CaCl$_2$ extracts prior to storage in a refrigerator at 4°C. The aqua regia extracts were made from 1 g of soil using 7.5 mL HCl and 2.5 mL HNO$_3$. Details on the extraction procedures can be found in the “Manual for the soil chemistry and fertility laboratory” (Van Ranst et al., 1999).

3.5 Chemical Analysis

3.5.1 Plant Samples

Approximately 0.2 g of each grounded sample was weighed in 50 mL microwave tubes using an analytical balance after which they were digested chemically using 2.5 mL HNO$_3$ and 2.5 mL hydrogen peroxide (H$_2$O$_2$) taken with a manual pipette. They were not tightly closed and left overnight in a fume cupboard.

These were then digested in a microwave oven at 1200W in three steps: firstly at 55°C for 10 minutes, then at 75°C for 10 minutes and finally at 100°C for 45 minutes. Thereafter the content of each tube was diluted to the 50 mL mark using distilled water. The cap was tightly fitted and the tube was then shaken to ensure homogeneity.

Approximately 20 mL from each tube was filtered using a filter paper into 20 mL test tubes. Thereafter, a 1/10 dilution with internal standard (Indium) was prepared from the filtered samples and the Se concentration in each diluted extract was determined by inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer Elan DRC-e, USA).

The remainder of the sample (filtered but not diluted) was then used to analyse for S by inductively coupled plasma optical emission spectrometry (ICP-OES, Varian Vista MPX, Palo Alto, USA).
3.5.2 Porewater samples

The frozen Rhizon samples were left to defrost in test tube racks after which their contents were transferred into labelled test tubes. Thereafter, a 1/10 dilution with internal standard (Indium) was prepared from the defrost samples and the Se concentration in each diluted sample was determined by inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer Elan DRC-e, USA).

3.5.3 Soil Extracts

From each of the soil extracts, approximately 20 mL was transferred into labelled test tubes. A 1/10 dilution was made from each test tube with Indium as internal standard and analysed for Se via inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer Elan DRC-e, USA). Considering the high concentration of the soil extracts spiked with selenate, a 1/10 dilution was first prepared with distilled water after which a further 1/10 dilution was made with Indium as internal standard before analysis. The remainder (for the aqua regia extracts) was analysed for S by inductively coupled plasma optical emission spectrometry (ICP-OES, Varian Vista MPX, Palo Alto, USA).

3.6 Quality Control of Data

Three plant reference materials were used. They included spruce needles (BCR 101), sea lettuce (BCR 279) and leak (own reference sample). The reported S for spruce needles after 12 months of storage is $1690 \pm 38 \text{ mg kg}^{-1}$, while the reported Se concentration in sea lettuce is $0.59 \pm 0.04 \text{ mg kg}^{-1}$. Their analysed Se and S concentrations are shown in Table 3.3.

<table>
<thead>
<tr>
<th>Reference Material</th>
<th>S/ mg kg$^{-1}$</th>
<th>Se/ mg kg$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spruce needles</td>
<td>1568 ± 12</td>
<td>0.058 ± 0.001</td>
</tr>
<tr>
<td>Sea lettuce</td>
<td>26383 ± 50</td>
<td>0.83 ± 0.09</td>
</tr>
</tbody>
</table>
As the experimental setup was not replicated, the analytical variability was assessed by analysing some randomly chosen plant samples 3 times for Se and S. Results, i.e. standard deviations on replicate analysis, are presented in Table 3.4.

Table 3.4. Results of replicate analysis of plant selenium and sulphur concentrations for assessing the analytical variability (average ± standard deviation, n = 3)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sample</th>
<th>S/ mg kg$^{-1}$</th>
<th>Se/ µg kg$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference_blank_selenite</td>
<td>T-0</td>
<td>3852 ± 133</td>
<td>16483 ± 967</td>
</tr>
<tr>
<td>Cow manure_selenite</td>
<td>T-0</td>
<td>3139 ± 12</td>
<td>1605 ± 107</td>
</tr>
<tr>
<td>Cow manure_selenate</td>
<td>T-0</td>
<td>4090 ± 28</td>
<td>20924 ± 3230</td>
</tr>
<tr>
<td>Compost_blank</td>
<td>T-1</td>
<td>3240 ± 85</td>
<td>545 ± 192</td>
</tr>
<tr>
<td>Lime_selenate</td>
<td>T-1</td>
<td>9761 ± 488</td>
<td>303775 ± 31559</td>
</tr>
<tr>
<td>Reference_blank</td>
<td>T-2</td>
<td>3441 ± 43</td>
<td>445 ± 61</td>
</tr>
<tr>
<td>Pig manure_selenate</td>
<td>T-2</td>
<td>4920 ± 51</td>
<td>33868 ± 1118</td>
</tr>
<tr>
<td>Lime_selenite</td>
<td>T-2</td>
<td>4044 ± 282</td>
<td>14362 ± 125</td>
</tr>
</tbody>
</table>
4. Results

4.1 Soil Characterisation

4.1.1 Soil pH

Table 4.1. Effect of amendments on soil pH (T-0: after 2 weeks of stabilisation, T-1 and T-2: after 1 and 2 months, respectively).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spike</th>
<th>T-0</th>
<th>T-1</th>
<th>T-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>Blank</td>
<td>6.6</td>
<td>7.5</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>6.6</td>
<td>7.5</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>6.6</td>
<td>7.5</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td>Barium selenate</td>
<td>6.8</td>
<td>7.5</td>
<td>7.4</td>
</tr>
<tr>
<td>Compost</td>
<td>Blank</td>
<td>6.7</td>
<td>7.6</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>6.7</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>6.8</td>
<td>7.5</td>
<td>7.6</td>
</tr>
<tr>
<td>Pig manure</td>
<td>Blank</td>
<td>6.6</td>
<td>6.9</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>6.6</td>
<td>6.9</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>6.8</td>
<td>6.7</td>
<td>7.1</td>
</tr>
<tr>
<td>Cow manure</td>
<td>Blank</td>
<td>7.3</td>
<td>8.1</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>7.2</td>
<td>7.8</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>7.2</td>
<td>7.9</td>
<td>8.1</td>
</tr>
<tr>
<td>Lime</td>
<td>Blank</td>
<td>7.0</td>
<td>7.6</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>7.0</td>
<td>7.8</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>7.0</td>
<td>7.8</td>
<td>7.8</td>
</tr>
</tbody>
</table>

In all soils, the initial soil pH was lower than the pH measured after one and two months. Applying pig manure resulted in a decrease in pH compared to the non-amended and compost-amended soil. Applying cow manure resulted in the highest increase of soil pH. Initially, the pH was already higher in the soil amended with cow manure, compared to the non-amended soil. Moreover, the pH still significantly increased between the first and the second month. When lime is used, the pH is also higher compared to the non-amended soil and also significantly increases with time, but it however already stabilises after 1 month. The pig manure amended soil spiked with selenite and the blank reference soils at the first sampling time had the lowest pH (6.6) while the soil amended with cow manure and spiked with selenite sampled after two months had the highest pH (8.7).
4.1.2 Electrical conductivity (EC)

Table 4.2. Effect of amendments on soil electrical conductivity (T-0: after 2 weeks of stabilisation, T-1 and T-2: after 1 and 2 months, respectively).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spike</th>
<th>T-0</th>
<th>T-1</th>
<th>T-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>Blank</td>
<td>430</td>
<td>526</td>
<td>471</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>465</td>
<td>509</td>
<td>455</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>353</td>
<td>347</td>
<td>310</td>
</tr>
<tr>
<td></td>
<td>Barium selenate</td>
<td>408</td>
<td>791</td>
<td>423</td>
</tr>
<tr>
<td>Compost</td>
<td>Blank</td>
<td>447</td>
<td>576</td>
<td>422</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>503</td>
<td>610</td>
<td>518</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>448</td>
<td>746</td>
<td>670</td>
</tr>
<tr>
<td>Pig manure</td>
<td>Blank</td>
<td>1282</td>
<td>1969</td>
<td>1526</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>1355</td>
<td>2300</td>
<td>1806</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>1327</td>
<td>2240</td>
<td>1808</td>
</tr>
<tr>
<td>Cow manure</td>
<td>Blank</td>
<td>1251</td>
<td>1143</td>
<td>1014</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>1206</td>
<td>1429</td>
<td>1070</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>1247</td>
<td>1419</td>
<td>1016</td>
</tr>
<tr>
<td>Lime</td>
<td>Blank</td>
<td>401</td>
<td>494</td>
<td>438</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>305</td>
<td>506</td>
<td>389</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>389</td>
<td>500</td>
<td>360</td>
</tr>
</tbody>
</table>

The EC in the soils was highest after one month. The pig manure treatments resulted in the highest EC, followed by the cow manure treatments, while the lime treatments resulted in the lowest EC. The compost treatment did not significantly increase the EC compared to the non-amended soil.

4.1.3 Organic Matter (OM)

Figure 4.1. Effect of amendments on soil organic content at T-0 (B = Barium, C=Compost, PM= pig manure, CM= cow manure).
The organic matter content significantly increased when cow manure, pig manure and compost were added, with cow manure resulting in the highest increase. Lime treatment did not significantly increase the organic matter content compared to the non-amended soil.

4.1.4 Cation Exchange Capacity (CEC)

![CEC Chart](image_url)

Figure 4.2. Effect on amendments on soil CEC at T-0 (B: Barium, C: Compost, PM: pig manure, CM: cow manure).

The compost, pig and cow manure amendments resulted in an increase in CEC compared to the non amended soils. The CEC was highest for the compost treatment and lowest for the lime amended soils.
### 4.2 Soil Samples

#### 4.2.1 Selenium

Aqua regia extracts are used for extracting (pseudo-) total trace element contents, so logically they always extracted the highest Se amounts (Table 4.3). As EDTA is a strong complexing agent, it also extracted more Se compared to CaCl$_2$. The highest CaCl$_2$- and EDTA-extractable concentrations were usually observed after 1 month of incubation. Overall, Se was more CaCl$_2$- and EDTA-extractable when selenate was used compared to when selenite was used, indicating a higher mobility of selenate compared to selenite. Overall, the use of compost, pig manure and

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spike</th>
<th>Aqua regia</th>
<th>CaCl$_2$</th>
<th>EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T-0</td>
<td>T-1</td>
<td>T-2</td>
</tr>
<tr>
<td>Reference</td>
<td>Blank</td>
<td>0.30</td>
<td>0.51</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>1.40</td>
<td>1.33</td>
<td>1.53</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>1.48</td>
<td>1.73</td>
<td>1.64</td>
</tr>
<tr>
<td></td>
<td>Barium selenate</td>
<td>1.58</td>
<td>2.58</td>
<td>1.60</td>
</tr>
<tr>
<td>Compost</td>
<td>Blank</td>
<td>0.54</td>
<td>0.44</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>1.44</td>
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<td>1.22</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
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<td>2.36</td>
<td>1.88</td>
</tr>
<tr>
<td>Pig manure</td>
<td>Blank</td>
<td>0.60</td>
<td>0.64</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>1.46</td>
<td>1.43</td>
<td>1.79</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>1.58</td>
<td>1.32</td>
<td>1.89</td>
</tr>
<tr>
<td>Cow manure</td>
<td>Blank</td>
<td>0.57</td>
<td>0.49</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>1.38</td>
<td>1.19</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>1.54</td>
<td>2.85</td>
<td>1.96</td>
</tr>
<tr>
<td>Lime</td>
<td>Blank</td>
<td>0.44</td>
<td>0.43</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>1.49</td>
<td>1.41</td>
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</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>1.37</td>
<td>1.72</td>
<td>1.48</td>
</tr>
</tbody>
</table>
Lime on soils spiked with selenate increased the CaCl$_2$- and EDTA-extractability of Se compared to the non-amended soil, whereas the use of cow manure decreased it. This was also the case for selenite, for which compost also seemed to have a reducing effect. Liming resulted in a slight increase of extractability in the selenate-spiked soils. Among the different amendments, use of pig manure resulted in the highest CaCl$_2$- and EDTA-extractability, and thus the highest mobility of Se.

### 4.2.2. Porewater samples

<table>
<thead>
<tr>
<th>Treatment</th>
<th>spike</th>
<th>T-0</th>
<th>T-1</th>
<th>T-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>Blank</td>
<td>6.2</td>
<td>6.5</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>264</td>
<td>366</td>
<td>239</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>4043</td>
<td>2204</td>
<td>2966</td>
</tr>
<tr>
<td></td>
<td>Barium selenate</td>
<td>2312</td>
<td>2139</td>
<td>1177</td>
</tr>
<tr>
<td>Compost</td>
<td>Blank</td>
<td>6.2</td>
<td>2.3</td>
<td>3.7</td>
</tr>
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<td></td>
<td>Sodium selenite</td>
<td>207</td>
<td>187</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>2684</td>
<td>2600</td>
<td>2793</td>
</tr>
<tr>
<td>Pig manure</td>
<td>Blank</td>
<td>21.2</td>
<td>12.6</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>280</td>
<td>187</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>1787</td>
<td>1410</td>
<td>1193</td>
</tr>
<tr>
<td>Cow manure</td>
<td>Blank</td>
<td>17.3</td>
<td>15.1</td>
<td>16.2</td>
</tr>
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<td></td>
<td>Sodium selenite</td>
<td>121.8</td>
<td>90.6</td>
<td>54.3</td>
</tr>
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<td></td>
<td>Sodium selenate</td>
<td>1230</td>
<td>751</td>
<td>401</td>
</tr>
<tr>
<td>Lime</td>
<td>Blank</td>
<td>7.4</td>
<td>6.2</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>232</td>
<td>206</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>3769</td>
<td>2260</td>
<td>1237</td>
</tr>
</tbody>
</table>

The results generally indicate a decrease of Se concentrations in pore water with time. In selenate spiked soils the Se concentration in pore water was much higher compared to the selenite spiked soils. In the spiked soils, the use of amendments resulted in lower Se concentrations in the porewater compared to the non-amended soil. Cow manure was the most effective in reducing Se concentrations in the porewater, whereas liming was the least effective. However, in the blank (non-spiked) soil, using pig and cow manure resulted in increased Se concentrations in the porewater. Porewater concentrations usually decreased with time in the spiked soils, except in the compost-amended soil spiked with selenate.
4.3. Se concentration in wheat

The plants grown on selenate spiked soils had the highest Se concentration followed by those grown on selenite spiked soils. The plants grown on non-spiked soils had the lowest Se concentration (Figure 4.3). The difference in Se concentration between the plants grown on soils spiked with selenate and varied from a minimum of 12 orders of magnitude high in the plants grown on cow and pig manure amended soils to over 40 in the compost treated soil plants.

The plants grown on cow and pig manure amended soils had a lower Se concentration compared to the plants grown on soils without amendments. However, the plants grown on compost amended soil had a much higher Se concentration compared to the plants grown on cow and pig manure amended soils. Though the Se concentrations in the plants grown on compost amended soils spiked with selenate did not differ significantly from the Se concentration in the plants grown on lime amended soils at the starting period, they were higher than the Se concentration in the plants grown on non-amended soils spiked with selenate. Over time, the Se concentration in plants at the starting period was higher than those grown on soils of age 1 and 2 months. An exception was in the plants grown on pig manure- and the non-amended soils which had the highest Se concentration when they were grown on soils of 1 and 2 months, respectively. A similar trend was observed for the plants grown on compost, lime and cow manure amended soils spiked with selenite which had the highest Se concentration at the starting period. Overall, the plants grown on non-amended soils spiked with selenite had the highest Se concentration compared to the plants grown on soils with amendments with the lowest Se concentration in plants grown on cow and pig manure amended soils.

The Se concentration in the plants grown on soils that were not spiked was highest in the compost and pig manure amended soils.
Figure 4.3. Wheat Se concentration grown on: (a) Compost amended soil, (b) pig manure amended soil, (c) Cow manure amended soil, (d) Lime amended soil, (e) reference treatment soil. The plants grown on soils spiked with selenate are plotted on a separate axis to those that were grown on non-spiked soils and soils spiked with selenite.
4.4 Sulphur

4.4.1 Wheat

Table 4.5. S concentration in wheat (mg kg\(^{-1}\)).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spike</th>
<th>T-0</th>
<th>T-1</th>
<th>T-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>Blank</td>
<td>3656</td>
<td>3347</td>
<td>3471</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>3758</td>
<td>3970</td>
<td>3862</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>6707</td>
<td>5839</td>
<td>8476</td>
</tr>
<tr>
<td></td>
<td>Barium Selenate</td>
<td>8189</td>
<td>5312</td>
<td>7997</td>
</tr>
<tr>
<td>Compost</td>
<td>Blank</td>
<td>3564</td>
<td>3301</td>
<td>3576</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>3644</td>
<td>3662</td>
<td>3950</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>11761</td>
<td>6784</td>
<td>8690</td>
</tr>
<tr>
<td>Pig manure</td>
<td>Blank</td>
<td>3525</td>
<td>3036</td>
<td>3452</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>3719</td>
<td>3732</td>
<td>3451</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>4682</td>
<td>6096</td>
<td>4884</td>
</tr>
<tr>
<td>Cow manure</td>
<td>Blank</td>
<td>3102</td>
<td>3249</td>
<td>3127</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>3131</td>
<td>3180</td>
<td>3032</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>4070</td>
<td>4060</td>
<td>3205</td>
</tr>
<tr>
<td>Lime</td>
<td>Blank</td>
<td>3623</td>
<td>3302</td>
<td>3421</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>4178</td>
<td>3919</td>
<td>4243</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>10193</td>
<td>10106</td>
<td>9598</td>
</tr>
</tbody>
</table>

The plants grown on soils spiked with selenate had a higher S concentration than plants grown on soils spiked with selenite. However, the S concentration in plants grown on soils spiked with selenite did not differ significantly from those grown on soils that were not spiked. On the other hand, the S concentration in the plants grown on soils amended with cow and pig manure spiked with selenate was low, while it was highest in the plants grown on soils amended with lime. A scatter plot of the plant Se against plant S concentration showing the influence of soil age is shown in Figure 4.4.
Figure 4.4. S concentration and Se concentration in wheat (T-0: after 2 weeks of stabilisation, T-1 and T-2: after 1 and 2 months, respectively).

The cloud of points around the S concentration of 4 µg kg$^{-1}$ are plants that were grown on the blank and selenite amended soils including those that were grown on the cow manure amended soil spiked with selenate. The scattered points represent the plants grown on the remaining selenate amended soils with the highest values seen always in the plants grown on lime amended soils. The high plant S concentrations corresponded with high plant Se concentrations in all the periods of soil ageing.

### 4.4.2 Soil

The soil S concentration determined from the aqua regia extract is shown in Table 4.6.

**Table 4.6. S concentration from aqua regia extracts (mg kg$^{-1}$).**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spike</th>
<th>T-0</th>
<th>T-1</th>
<th>T-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>Blank</td>
<td>268</td>
<td>298</td>
<td>219</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>286</td>
<td>276</td>
<td>215</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>270</td>
<td>281</td>
<td>228</td>
</tr>
<tr>
<td></td>
<td>Barium selenate</td>
<td>255</td>
<td>290</td>
<td>227</td>
</tr>
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<td>Compost</td>
<td>Blank</td>
<td>405</td>
<td>402</td>
<td>343</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>496</td>
<td>354</td>
<td>301</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>366</td>
<td>372</td>
<td>378</td>
</tr>
<tr>
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<td>779</td>
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<td>762</td>
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<td>Sodium selenate</td>
<td>611</td>
<td>569</td>
<td>506</td>
</tr>
<tr>
<td>Cow manure</td>
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<td>427</td>
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<td>441</td>
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<td>527</td>
<td>740</td>
<td>548</td>
</tr>
<tr>
<td>Lime</td>
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<td>280</td>
<td>210</td>
<td>228</td>
</tr>
</tbody>
</table>
The non-amended and the lime amended soils had the lowest soil S concentration over the entire period. However, the S concentration in the cow and pig manure amended soils was higher than for the soil amended with compost.

The relation between the soil S concentration and the Se content of plants is shown in the Figure 4.5.

![Figure 4.5. Soil sulphur and plant selenium concentration (T-0: after 2 weeks of stabilisation, T-1 and T-2: after 1 and 2 months, respectively).](image)

The points along the horizontal axis represent plants grown on blank and selenite spiked soils including those grown on the cow and pig manure selenate treatment grown plants. The other points represent plants grown on selenate amended soils. A low soil S with the corresponding highest value for the plant Se concentration was observed for the reference selenate and lime selenate treatments.

### 4.5 Dry weight

The plant dry weight was on average highest for the plants grown on soil of age 1 month and lowest for those grown on the soils at the starting period.
Table 4.7. Plant dry weight (g).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spike</th>
<th>T-0</th>
<th>T-1</th>
<th>T-2</th>
</tr>
</thead>
<tbody>
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<td>Reference</td>
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<td>0.77</td>
<td>1.31</td>
<td>0.79</td>
</tr>
<tr>
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<td>Sodium selenite</td>
<td>0.79</td>
<td>1.13</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>0.74</td>
<td>1.01</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>Barium selenate</td>
<td>0.55</td>
<td>1.25</td>
<td>0.87</td>
</tr>
<tr>
<td>Compost</td>
<td>Blank</td>
<td>0.89</td>
<td>1.03</td>
<td>0.89</td>
</tr>
<tr>
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<td>Sodium selenite</td>
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<td>Sodium selenate</td>
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<td>1.22</td>
<td>0.84</td>
</tr>
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</tr>
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</tr>
<tr>
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<td>Sodium selenate</td>
<td>0.99</td>
<td>1.10</td>
<td>0.87</td>
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<tr>
<td>Cow manure</td>
<td>Blank</td>
<td>1.04</td>
<td>1.18</td>
<td>0.95</td>
</tr>
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<td>Sodium selenite</td>
<td>1.14</td>
<td>1.04</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>0.91</td>
<td>1.50</td>
<td>1.05</td>
</tr>
<tr>
<td>Lime</td>
<td>Blank</td>
<td>0.77</td>
<td>1.05</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>Sodium selenite</td>
<td>0.71</td>
<td>1.16</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>Sodium selenate</td>
<td>0.66</td>
<td>1.08</td>
<td>0.88</td>
</tr>
</tbody>
</table>

A plot of plant Se concentrations against plant dry weight is shown in Figure 4.6.

![Plot of plant Se concentrations against plant dry weight](image)

**Figure 4.6.** Plant dry weight and Se concentration (T-0: after 2 weeks of stabilisation, T-1 and T-2: after 1 and 2 months, respectively).

From Figure 4.6, the points along the plant horizontal axis represent plants grown on blank and selenite spiked soils including those grown on the cow and pig manure amended soils spiked with selenate. The other points represent plants grown on non-amended sodium selenate and barium selenate plants, including plants grown on compost and lime amended
soils spiked with selenate. However, the Se concentration in the plant was higher at the starting period with the lowest amount of dry weight and lowest in the plants grown on soil of age one month though with the highest amount of dry matter.

A plot of the soil Se concentration from aqua regia, EDTA, and CaCl₂ extracts with the total Se content of wheat is shown in Figure 4.7.

Figure 4.7. Correlation of soil-extractable Se and total Se content of wheat.

The correlation coefficient was higher for the ammonium acetate-EDTA extract and the CaCl₂ extract and lowest for the aqua regia extract.
5. Discussion

5.1 Soil Characterisation

5.1.1 pH

From Table 4.1, it can be observed that there is an impact of amendments and soil age on pH. This will therefore have an effect on Se bioavailability and subsequent uptake by plants (Fordyce, 2005). According to the USDA descriptive ranges for pH in soils (appendix 1), almost all soils at the starting period had a neutral pH though with different values within the range. On average, these became slightly alkaline after the first month except for the pig manure amended soil which remained neutral. After two months, the pig manure and the non-amended (reference) soils remained neutral. The compost and the lime amended soils remained slightly alkaline while for the cow manure amended soil, the blank and selenate spiked soils became moderately alkaline and the selenite spiked soil became strongly alkaline. The increased pH due to liming was responsible for the high pH in the lime amended soils compared to the non amended soils. Among the organic amended soils, the cow manure amended soil had a high pH. This variability in pH can be attributed to changing microbial activities in the soil after sampling and during stabilisation. The pig manure resulted in soil acidification compared to the non amended soil while the cow manure made the soil to become alkaline.

5.1.2 Electrical Conductivity (EC)

The chemical property which indicates the amount of soluble salts, EC, was highest for pig and cow manure amended soils. This is mainly due to the additives such as salt, Cu and Zn ions incorporated into livestock and poultry feeds to shorten the duration of breeding which finally end up in the manure (Cang et al., 2004; Nicholson et al., 1999). This finding is further backed by Hoa et al. (2008) who in a leaching experiment done on soils with and without pig manure amendments, reported high leaching rates for pig manure amended sandy loam soils indicated by a high value for the electrical conductivity due to the Cu and Zn ions it contained. The decomposition of organic matter in the organic amended soils may also introduce large amounts of low-molecular weight organic acids to soils (Baziramakenga and Simard, 1998) which may also increase the EC.
5.1.3 Organic matter and CEC

Soil organic matter increases CEC. In the surface horizon of mineral soils, soil organic matter is responsible for 25-90% of the total CEC (Van Dijk, 1971). The organic content of the compost, cow and pig manure amended soils was therefore responsible for the high CEC (on average higher than 12.3 cmol+ kg⁻¹) in these treatments compared to the reference and lime amended soils. The highest value in for the CEC (12.88 meq 100 g⁻¹) was observed in the compost selenate treatment. Though there are great differences in the biochemical composition of the organic treatments, Oorts et al. (2003) reported that organic matter inputs have a negligible effect on the CEC of soil organic matter in ferric lixisols.

5.2 Soil extracts

There is a great variability in the CaCl₂- and EDTA-extractable Se concentration. Zhao et al. (2005) reported that there is a great impact of soil physical and chemical factors on the Se extracted from soils, which included CaCO₃, pH, silt content, exchangeable quantity, clay content, available iron and organic matter. Though these extractions are meant to indicate the likely bioavailability in seleniferous soils, Davies (1980) reported that the bioavailable Se in its different forms is difficult to separate and identify due to the extremely complex physicochemical properties of soils.

Considering the fact that Se in an aqua-soluble form could indicate the bioavailable Se, CaCl₂ being a weak aqueous extract can thus be considered to represent the likely bioavailability. But, this is contradicted by Zhao et al., (2005) for the case of Se deficient soils due to the weak buffer capability of the aqueous solution, easy change of pH and weak extraction capability of water. Aqua regia on the other hand being a stronger extract indicated the highest levels of Se while the CaCl₂ extracts had the lowest Se concentration. This indicates that CaCl₂ is a weak extract. This is supported by Zhao et al (2005) who reported that the content of extractable Se with water from high Se level soils was not so high. In their study, the total Se content of the soil was 23.53 mg kg⁻¹, but the water extractable Se was 5.64 mg kg⁻¹ in the Se-toxic soils of Ziyang County, which occupied only 0.24% of the total soil Se.
5.3 Porewater samples

The pore water extractable Se was found to decrease with time. This shows that the pore water soluble Se, indicating the likely bioavailability, would decrease with time from its growth medium given that it will be taken up by plants. The high selenate concentration compared to the selenite concentration is due to the fact that selenate is weakly adsorbed on the soil while selenite on the other hand reacts strongly with the soil (Yläranta 1983a). Selenate therefore will be leached more easily compared to selenite. The lowest values for the cow manure at the starting period (selenite 121 µg L⁻¹ and selenate 1230 µg L⁻¹) are in conformity with the findings of Dhillon et al., (2010) who reported that incubating naturally occurring selenium forms with organic amendments led to a substantial decrease (20%-26%) of easily available (water-soluble and extractable) forms of Se and a corresponding increase of (13%-62%) in the less available (organic matter and metal oxide bound) Se forms. The same low values in the subsequent periods are in conformity with the binding of Se by cow manure. The high value for the compost amended soil spiked with selenate of 2684 µg L⁻¹, 2600 µg L⁻¹ and of 2793 µg L⁻¹ in the starting, second and final periods of soil ageing, respectively, indicate that the selenate spiked to compost was weakly bound. A much higher selenate concentration was observed for the reference selenate treatment (4043 µg L⁻¹) and the lime amended soil spiked with selenate (3769 µg L⁻¹) at the starting period indicate that the selenate in these soils was also weakly bound.

The Se concentrations in the blank treatments indicated the background concentrations which were lowest for the reference, compost and lime blank soils with values of 6.2 µg L⁻¹, 6.2 µg L⁻¹ and 7.4 µg L⁻¹, respectively, at the starting period. It was highest for the pig manure blank treatment (21.2 µg L⁻¹) followed by the cow manure blank treatment (17.3 µg L⁻¹) in the starting period. This is an indication that the cow and pig manure contained Se. Aside the effect of the organic treatments, variation in pore water metal concentrations may also be related to the moisture regime of the soil (Du Laing et al., 2007).

5.4 Influence of soil amendments on Se uptake by wheat plants

The highest Se concentration was observed in the plants grown on selenate spiked soils due to the high bioavailability of selenate compared to selenite and hence easily taken up by plants. The highest plant Se concentration at the starting period was seen in the plants grown on the reference, lime and compost amended soils, spiked with selenate. The high value for
the compost amended soil confirms the fact that compost weakly binds selenate, resulting in a high pore water Se concentration. This value, of same order of magnitude as the value in the lime amended selenate spiked soil, was higher than that of the reference (non-amended) soils spiked with selenate. A similar trend was also observed for the reference and lime amended selenite spiked soils at the starting period. Liming therefore can be concluded to increase Se bioavailability. This is in conformity with the study by Ylaränta (1983b, 1990) who reported that lime applied to soils to ameliorate soil acidity can be assumed to reduce Se sorption by soil and therefore increase its uptake by plants.

The Se concentration in the plants grown on non-amended soils spiked with selenate was higher compared to those grown on soils that were spiked by barium selenate. This is due to the low solubility of barium selenate compared to sodium selenate. Among the plants grown on soils spiked with selenite, those grown on pig and cow manure amended soils had the lowest Se concentration. The cow manure selenite spiked soil had the lowest plant Se concentration at the starting period. This coincided with the corresponding porewater concentration which indicates that cow manure results in the removal of Se from the soil solution. This is due to the interaction of Se with organic compounds making them less mobile as indicated by the increase in insoluble Se fractions (Pezzarossa et al., 2007) such as fixation by organometallic complexes. This therefore reduces the amount which is available for plant uptake and thus the amount accumulated in the plant especially in the seeds (Johnson, 1991). A similar phenomenon of metal binding by organic matter (Dhillon et al., 2010) may have resulted in the low plant Se concentration in the pig manure treatment. The higher Se concentration in the plants grown on the compost and pig manure blank treatments at the starting period compared to the non-amended soil is due to the back ground concentrations generated by the amendments. Though the pig manure originally had a higher background concentration, the higher value for the compost may be due to the release of low molecular-weight Se compounds during the decomposition of the compost which could serve as a source of Se for plant uptake (Zawislanski and Zavarin 1996).

The variability within the time periods can be attributed to the effects of organic carbon amendments on Se (im) mobilisation in soils and also due to the wetting and drying of soil which promoted rapid transformation of \( \text{SeO}_4^{2-} \) into other forms, such as \( \text{SeO}_3^{2-} \) and organic Se that are adsorbed to soil surfaces (Neal and Sposito 1991).
Long-term field studies are therefore needed to monitor Se transformation in soils amended with seleniferous crop residues and organic wastes (Ajawa et al., 2005).

5.5 Soil and plant sulphur relation to Se uptake by wheat plants

In wheat, the main form of Se uptake is selenate through the sulphate pathway (Li et al. 2008). This relation can be seen on Figure 4.4, where high plant S concentrations in the plants grown on soils spiked with selenate correspond with a high plant Se concentration. This therefore confirms the synergistic relationship between Se and S (Mikkelsen et al., 1988a, b) who observed that elevated concentrations of Se in the root zone might increase the plant S accumulation when sulphate concentrations are low in the root zone. An exception among the plants grown on soils spiked with selenate is seen for those grown on the cow and pig manure amended soils. From Table 4.6, these are seen to have the highest values for S along with compost treatment. But, on the contrary, the plant Se concentrations were the lowest with exception in the plants grown on compost amended soil. It can thus be concluded that the compost contained S which was in a form readily bioavailable for plant uptake, whereas the cow and pig manure did not. The lime and reference treatments having the lowest value for soil S concentration had higher S concentrations in the plants grown on them. The S in these treatments can thus be concluded to be readily bioavailable. This is seen by their high plant Se concentrations which can be attributed to the sulphate uptake route of selenate (Terry et al., 2000).

From Table 4.5, it can be seen that S concentration of plants grown in the selenite spiked soils and blank treatments were almost of same order of magnitude. Se concentration of similar order of magnitude for the same treatments can be seen in Figure 4.4 which corresponds to these low S concentrations. This suggests that the Se uptake mechanism in these soils is different than for the selenate spiked soils. This confirms the fact that selenite in soils has a low bioavailability and uptake by plants is via passive diffusion (Arvy, 1993).

5.6 Plant growth and Se uptake

Plants vary considerably in their physiological response to excess Se in the growth medium (Terry et al., 2000). From Figure 4.6, it is evident that Se bioavailability was high at the
starting period in the selenate spiked soils, especially for the reference, compost and lime amended soils spiked with selenate. This became lower after 1 month of ageing and increased after 2 months of ageing depicted by the concentrations in wheat. This may probably be linked with the alternate wetting and drying of the soil which promoted rapid transformation of selenate into other forms, such as selenite and organic Se that were adsorbed onto soil surfaces (Neal and Sposito 1991). These probably became oxidised again increasing the bioavailability. The high uptake is seen to have an adverse effect on dry matter production. Plants grown on selenate spiked soils at the starting period had the highest Se concentration and the lowest amount of biomass, while those grown on soils after 1 month had the lowest plant Se concentration and a high amount of biomass. This confirms the fact that in wheat dry matter production is affected by high plant Se concentrations (Rani et al., 2005). In the plants grown on the blank and selenite spiked soils, the effect on dry matter production was not observed although a variability of Se uptake with soil age was observed.

5.7 Environmental Relevance

Figure 4.7 shows that the EDTA- and CaCl₂-extractable Se reflects the plant Se concentration better than the aqua regia extract. Thus, from the total amount of Se applied to the soil, plants can only take up part during their period of growth. This uptake was high for the plants grown on non-amended, compost and lime amended soils spiked with selenate with an uptake rate of over 40% of the applied Se. However, the plants grown on lime amended soil spiked with selenate had the highest rate of uptake (48%) of the applied Se. In such soils, the risk of Se accumulation will be lower. On the other hand, the plants grown on pig and cow manure amended soils spiked with selenate had the lowest rate of Se uptake of 4 and 2%, respectively. In the plants grown on soils spiked with selenite, the uptake was over 2% in the non-amended, lime and compost amended soils and 1% in the cow manure amended soil. In the pig manure amended soil and the non-spiked soils (blanks), the uptake was less than 1%. In such soils with low uptake of the Se applied to soil, the Se will therefore accumulate in the soil. Under saturated conditions, the Se concentration in pore water from theses soils where Se has accumulated will be high due to leaching. This may move through the pores in the soil and finally end up in groundwater. Overtime, the concentration in ground water may increase and will be of great threat to communities which depend on ground water for their domestic water supply.
6. Conclusion and recommendation

The results of this study show that selenate is the major form through which plants take up Se from the soil. This varied among the plants grown on non-amended (reference), organic and inorganic amended soils. Amongst the organic amended soils, the plants grown on compost amended soil had the highest Se concentration, while the plants grown on cow manure amended soil had the lowest concentration with selenium applied in the form of selenate. In the non-amended soils (reference treatment), the high plant Se concentration in the sodium selenate treatment compared to the barium selenate treatment shows that the former has a higher bioavailability in soils and therefore higher uptake by plants. In wheat, liming results in a higher uptake of Se applied to the soil in form of selenate. On the other hand, the plants grown on soils spiked with selenite had a much lower Se concentration compared to those grown on soils spiked with selenate. A high Se concentration was seen in plants grown on non-amended (reference), compost and lime amended soils, while it was lowest in the plants grown on cow and pig manure amended soils.

There is a risk of environmental contamination especially of ground water when Se is applied to the soil in the form of selenite and on soils which are amended with cow and pig manure irrespective of the form in which Se is applied to the soil. However, Se supply from organic amendments also supplement the amount available for plant uptake especially when compost and cow manure are used as soil amendment. Considering the fact that only the seeds from wheat are used in production of food products, the concentration in the leaves cannot be used to determine the risk to humans upon consumption, as the concentration which ends up in the seeds is not known. However, it may be used to assess the amount available for plant uptake.

In order to fully monitor the Se transformation in soils amended with seleniferous crop residues and organic wastes, long-term field studies are needed. Thus, considering the fact that this was an exploratory study, in subsequent experiments, a longer time period (greater than 3 months) should be used along with replicates to better understand the effects of organic and inorganic amendments on Se mobility and bioavailability in soils.

The low Se uptake by the plants grown on cow and pig manure amended soils irrespective of the form in which Se is applied to the soil warrants further research of their effects on Se availability and mobility. Since these were characterised by high values of EC, there could...
possibly be some dissolved constituents from these treatments which were responsible for the low Se uptake by plants.
References


Moxon, A.L., 1937. Alkali Disease or Selenium Poisoning, Bulletin 311, South Dakota Agricultural Experimental Station, Vermillion.


## Appendix

Appendix 1. Descriptive ranges for pH in soils

<table>
<thead>
<tr>
<th>pH</th>
<th>Value</th>
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<tbody>
<tr>
<td>Ultra acid</td>
<td>&lt; 3.5</td>
</tr>
<tr>
<td>Extremely Acid</td>
<td>3.5-4.4</td>
</tr>
<tr>
<td>Very strongly acid</td>
<td>4.5-5.0</td>
</tr>
<tr>
<td>Strongly acid</td>
<td>5.1-5.5</td>
</tr>
<tr>
<td>Moderately acid</td>
<td>5.6-6.0</td>
</tr>
<tr>
<td>Slightly acid</td>
<td>6.1-6.5</td>
</tr>
<tr>
<td>Neutral</td>
<td>6.6-7.3</td>
</tr>
<tr>
<td>Slightly alkaline</td>
<td>7.4-7.8</td>
</tr>
<tr>
<td>Moderately alkaline</td>
<td>7.9-8.4</td>
</tr>
<tr>
<td>Strongly alkaline</td>
<td>8.5-9.0</td>
</tr>
<tr>
<td>Very strongly alkaline</td>
<td>&gt; 9.0</td>
</tr>
</tbody>
</table>

Source: USDA soil quality – agronomy technical note N0. 8